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A Short Chemoenzymatic Synthesis of (+)-Narciclasine

David Gonzalez,[i] Theodore Martinot,[ii] and Tomas Hudlicky*,[iii]

Chemistry Department, University of Florida, Gainesville, FL 32611

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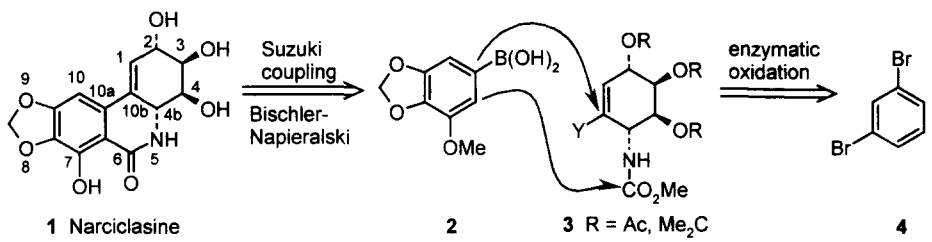
Abstract: The title alkaloid has been synthesized in eight operations from dibromobenzene and *o*-vanillin, via enzymatic oxidation of the former compound, Suzuki coupling and a Bischler-Napieralski type cyclization as the key transformations.

Narciclasine (**1**), the last of the antitumor Amaryllidaceae alkaloids to yield to total synthesis,[1] is found in the extract of *Lycoris radiate*,[2] *Pancratium litorale*,[3] and *Pancratium maritimum*,[4] as well as in several *Narcissus* species.[5] Of the four well-known compounds (pancratistatin, 7-deoxypancratistatin, lycoricidine, and narciclasine) that have attracted attention as potential antineoplastic agents,[6] narciclasine is the only one whose mode of activity has been briefly investigated.[9c]



Scheme 1. Design of narciclasine synthesis.

The closely related and more abundant lycoricidine lacks the 7-hydroxyl group believed to be important in the biological activity of narciclasine.[2] Pancratistatin (1*R*-hydroxy-10bαH derivative of **1**), and 7-deoxypancratistatin are related in the same way, but the latter substance is about 10 times less active than the former in identical cell line screens.[7] An attempt to convert the more abundant congeners to pancratistatin by hydration of the C1-C10b olefin was unsuccessful.[8] To date a truly practical synthesis of **1** or its congeners has not materialized. Thus, the supply-and-demand issue of antitumor screening of these alkaloids has not been alleviated. Several syntheses of these alkaloids have been reported;[1,9-11] and the various synthetic strategies were recently summarized in two excellent reviews.[12] In 1995, a chemoenzymatic strategy from this laboratory led to the first (and, at 13 steps, still the shortest) enantioselective synthesis of pancratistatin.[9b-c] In subsequent studies, we addressed several major improvements to shorten further the synthetic sequence and to provide a fully general and highly efficient route to all four alkaloids. In this report, we describe a brief route (eight operations) to the title compound via an enzymatic dihydroxylation of *m*-dibromobenzene.

m-Dibromobenzene was subjected to the whole-cell fermentation with *E. coli* JM109(pDTG601A), an organism developed by Gibson[13-15] for the overexpression of toluene dioxygenase (TDO), whose structure has recently been solved.[16] Biooxidation yielded the new metabolite **5** (4 g/L, >99% ee) that presents unique symmetry and two chemically different vinylic bromine atoms (C3 bromine is hydrogen bonded to C2 OH), Scheme 2. The

[i] davidg@ufl.edu
[ii] theox@ufl.edu
[iii] hudlicky@chem.ufl.edu

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rotation of the symmetry axis that takes place during the transformation of **4** into **5**, positions the bromine atoms in different planes.[§] This symmetry rotation will later allow the selective incorporation of the aromatic fragment of narciclasine. Diol **5** was transformed in a one-pot operation to bicyclic oxazine **6** in 70% yield, as shown in Scheme 2. Reduction of this material under Keck's conditions[17,18] to yield the conduramine oxidation state as previously reported,[10c-d,19] gave predominantly the fully dehalogenated conduramine derivative **7b**.

We studied the possibility of reducing the oxazine to unsaturated ketone **8**. Tributyltin hydride or *tris*-trimethylsilylsilane (TTMSS) are suited for this transformation but cannot be applied to oxazine **6** since overreduction of the vinylic bromine is unavoidable under such conditions. Conversely, $Mo(CO)_6$[20] cleanly reduced dibrominated oxazine **6** to the corresponding bromo ketone **8** with concomitant (and interesting) cleavage of the acetonide protecting group. With this result in hand, we explored the possibility of directed hydride reduction by means of $Zn(BH_4)_2$[21] or $Na(AcO)_3BH_4$.[22] To date this reaction has provided triol **9** in only a disappointing 10% diasteromeric excess (HPLC). To circumvent the problem of overreduction of the bromine atom, we decided to couple the aromatic portion of the alkaloid directly to oxazine **6** and postpone the bridge opening to a later stage in the synthesis. Surprisingly, oxazine **10** was resistant to aluminum amalgam reduction, while stronger reducing agents led to fully saturated products. This result closed the direct reduction pathway to the α-hydroxy compound; therefore, we chose to transform **10** into unsaturated ketone **12** with TTMSS.

Interestingly, a considerable amount of **11** (10-15%) was formed during Suzuki coupling of bromide **6** with borate **2** (prepared from *o*-vanillin in four steps and 20% overall yield[23]) probably through a Pd insertion-type mechanism. Guided by this observation we decided to add acetonitrile and $Mo(CO)_6$ directly to the Suzuki reaction mixture after the coupling was finished. Heating of this mixture for 10 hours afforded ketone **11** in 45% yield, reaching the advanced intermediate **11** in only three steps from *m*-dibromobenzene.

In order to set the stereochemistry at C2 (narciclasine numbering), we applied the known $NaBH_4$ reduction followed by Mitsunobu inversion sequence as reported by Chida in his lycoricidine preparation.[10b,c] This procedure gave cleanly the desired α-benzoate **14** in 50% (from **12**).

A modification of the Bischler-Napieralski reaction reported by Banwell[24a] and applied with success by the same author in simplified models of phenanthridone alkaloids,[24b] was chosen to close ring B of the target after the required manipulation of acetonide **14** into diacetate **15** (30% over the three steps). Finally, removal of the ester and methyl ether protective groups in **16** (Amberlyst basic, MeOH and LiCl, DMF respectively)[25] rendered synthetic narciclasine (**1**) whose ^1H NMR and optical rotation matched the reported data for the alkaloid.[26]

Overall, we have completed a total synthesis of narciclasine in 12 steps carried out as only 8 individual chemical operations from *m*-dibromobenzene (14 from *o*-vanillin). A complete description of the routes depicted in Scheme 2, with focus on the results obtained in the reduction of the oxazine system under different conditions as well as the $Zn(BH_4)_2$ reduction of dihydroxy ketones, will appear in an upcoming full account.

Acknowledgment

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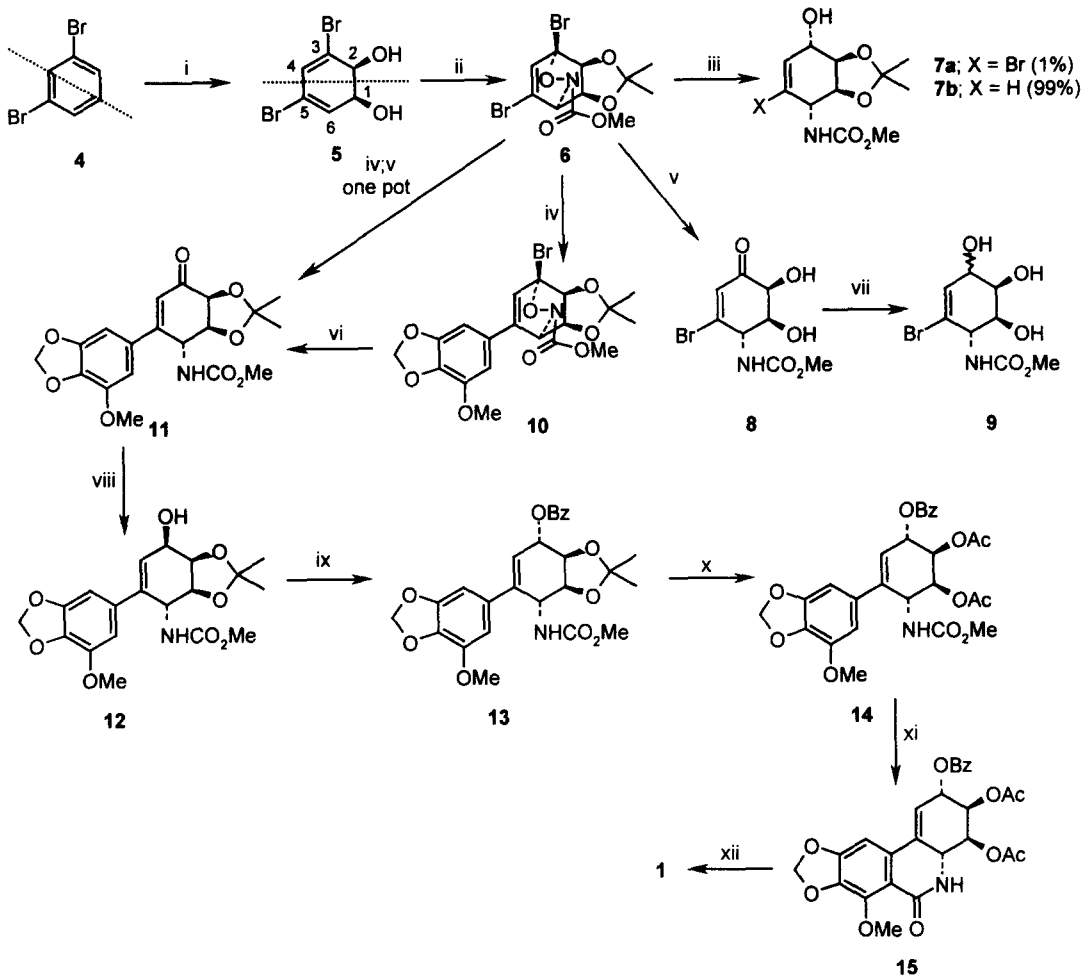

i) *E. coli* JM109 (pDTG601A), 4g/L; ii) DMP, acetone, TsOH, rt; then $NHCO_2Me$, $NaIO_4$, rt, 70%; iii) Al(Hg), THF, 80%; iv) borate **2**, $Pd(PPh_3)_4$, aq. Na_2CO_3, PhH, reflux, 30%; v) $Mo(CO)_6$, MeCN-H_2O, reflux, 75%; vi) TTMSS, AIBN, PhH, reflux, 80%; vii) $Zn(BH_4)_2$, DME, -10 °C, 70%; viii) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, 80%; ix) BzOH, Bu_3P, DEAD, THF, rt, 65%; x) Dowex 50X8-100, MeOH, rt; then Ac_2O, py, DMAP, rt, 70%; xi) Tf_2O, DMAP, CH_2Cl_2, 0 °C, 40%; xii) Amberlyst A21, MeOH, rt; then LiCl, DMF, 120 °C, 20%.

Scheme 2. Summary of the narciclasine synthesis.

References and notes

§ We can designate these planes as "prochemotopic", in addition to "proenantiotopic", since the vinylic bromine atoms will become chemically different after the next operation. The Diels-Alder cycloaddition transformed the bromine at C3 into an allylic one.

1. a) Rigby, J. H.; Mateo, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 12655; b) Keck, G. E.; Wager, T. T.; Rodriguez, F. "A concise route to the Amaryllidaceae alkaloids (+)-lycoricidine and (+)-narciclasine via a radical cyclization approach"; 216th ACS National Meeting, Boston, 1998.
2. Okamoto, T.; Torii, Y.; Isogai, Y. *Chem. Pharm. Bull.* **1968**, *16*, 1860.
3. Pettit, G. R.; Gaddamidi, V.; Cragg, G. M.; Herald, D. L.; Sagawa, Y. *J. Chem. Soc., Chem. Commun.* **1984**, 1693.

4. Abou-Donia, A. H.; De Giulio, A.; Evidente, A.; Gaber, M.; Habib, A.; Lanzeta, R.; Seif El Din, A. *Phytochemistry* **1991**, *30*, 3445.
5. a) Piozzi, F.; Fugante, C.; Mondelli, R.; Ceriotti, G. *Tetrahedron* **1968**, *24*, 1119; Evidente, A. *Planta Med.* **1991**, *57*, 293.
6. a) Pettit, G. R.; Gaddamidi, V.; Herald, D. L.; Singh, S. B.; Cragg, G. M.; Schmidt, J. M.; Boettner, F.; Williams, M.; Sagawa, Y. *J. Nat. Prod.* **1986**, *49*, 995; b) Ceriotti, G. *Nature* **1967**, *11*, 595; c) Jimenez, A.; Santos, A.; Alonso, G.; Vazquez, D. *Biochem. Biophys. Acta* **1976**, *425*, 342.
7. Pettit, G. R.; Hudlicky, T., unpublished observations.
8. Pettit, G. R.; Melody, N.; O'Sullivan, M.; Thompson, M. A.; Herald, D. L.; Coates, B. *J. Chem. Soc., Chem. Commun.* **1994**, 2725.
9. **Pancratistatin**: a) Danishefsky, S.; Lee, J. Y. *J. Am. Chem. Soc.* **1989**, *111*, 4829; b) Tian, X.; Hudlicky, T.; Konigsberger, K. *J. Am. Chem. Soc.* **1995**, *117*, 3643; c) Hudlicky, T.; Tian, X.; Konigsberger, K.; Maurya, R.; Rouden, J.; Fan, B. *J. Am. Chem. Soc.* **1996**, *118*, 10752.; d) Trost, B. M.; Pulley, S. R. *J. Am. Chem. Soc.* **1995**, *117*, 10143; e) Doyle, T. J.; Hendrix, M.; Vanderveer, D.; Javanmard, S.; Haseltine, J. *Tetrahedron* **1997**, *53*, 11153; f) Magnus, P.; Sebhat, I. K. *J. Am. Chem. Soc.* **1998**, *120*, 5341; g) Magnus, P.; Sebhat, I. K. *Tetrahedron* **1998**, *54*, 15509.
10. **Lycoricidine**: a) Paulsen, H., Stubbe, M. *Liebigs Ann. Chem.* **1983**, 535; b) Chida, N.; Ohtsuka, M.; Ogawa, S. *Tetrahedron Lett.* **1991**, *32*, 4525; c) Chida, N.; Ohtsuka, M.; Ogawa, S. *J. Org. Chem.* **1993**, *58*, 4441; d) Hudlicky, T.; Olivo, H. F. *J. Am. Chem. Soc.* **1992**, *114*, 9694; e) Hudlicky, T.; Olivo, H. F.; McKibben, B. *J. Am. Chem. Soc.* **1994**, *116*, 5108; f) Martin, S. F.; Tso, H.-H. *Heterocycles* **1993**, *35*, 85; g) **enantiomer:** Keck, G. E.; Wager, T. T. *J. Org. Chem.* **1996**, *61*, 8366.
11. **7-deoxypancratistatin**: a) Tian, X.; Maurya, R.; Konigsberger, K.; Hudlicky, T. *Synlett* **1995**, 1125; b) ref. 9c; c) Keck, G. E.; Mc.Hardy, S. F.; Murry, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 7289; d) Chida, N.; Jitsuoka, M.; Yamamoto, Y.; Ohtsuka, M.; Ogawa, S. *Heterocycles* **1996**, *43*, 1385; e) Keck, G. E.; Wafer, T. T; McHardy, S. F. *J. Org. Chem* 1998, *63*, 9164.
12. a) Hoshino, O. In *The Alkaloids*; Cordell, G. A., Ed.; Ac. Press: San Diego, 1998; Vol. 51, p 323; b) Polt, R. In *Organic Synthesis: Theory and Applications*; Hudlicky, T., Ed.; JAI Press: 1997; Vol. 3, p 109.
13. Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.
14. Boyd, D. R. *Nat. Prod. Reports* **1998**, 309.
15. Hudlicky, T.; Gibson, D. T.; Gonzalez, D. *Aldrichimica Acta* **1999**, in press.
16. Kauppi, B.; Lee, K.; Carredano, E.; Parales, R. E.; Gibson, D. T.; Ekiund, H.; Ramaswamy, S. *Structure* **1998**, *6*, 571.
17. Keck, G. E.; Fleming, S.; Nickell, D.; Weider, P. *Synth. Commun.* **1979**, *9*, 281.
18. Keck, G. E.; Mc.Hardy, S. F.; Wager, T. T. *Tetrahedron Lett.* **1995**, *36*, 7419.
19. Hudlicky, T.; Olivo, H. F. *Tetrahedron Lett* **1991**, *32*, 6077.
20. a) Olivo, H. F.; Hemenway, M. S.; Hartwig, A. C.; Chan, R. *Synlett* **1998**, 247; b) Ritter, A. R.; Miller, M. J. *J. Org. Chem.* **1994**, *59*, 4602.
21. a) Paquette, L. A. *Encyclopedia of Reagents for Organic Synthesis*; J. Wiley: New York, 1995; Vol. 8, 5536; b) Narashimhan, S.; Balakumar, R. *Aldrichimica Acta* **1998**, *31*, 19.
22. Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, *93*, 1307.
23. For the preparation of the precursory aryl bromide see: a) Shirasaka, T.; Takuma, Y.; Imaki, N. *Synth. Commun.* **1990**, *20*, 1223; b) Dallacker, F.; Sanders, J. *Chem. -Ztg.* **1984**, *108*, 186.
24. a) Banwell, M. G.; Bissett, B. D.; Busato, S.; Cowden, C. J.; Hockless, D. C. R.; Holman, J. W.; Read, R. W.; Wu, A. W. *J. Chem. Soc., Chem. Commun.* **1995**, 2551; b) Banwell, M. G.; Cowden, C. J.; Gable, R. W. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3515.
25. We thank Dr. S. Pulley (U. of Missouri) and Prof. B. M. Trost (Standford U.) for supplying the correct conditions for the final demethylation. In the original paper by these authors (ref. 9d) the use of LiI is reported.
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